14. Zone-Forming Fungi

Experiment MA-147*

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ABSTRACT

Streptomyces levoris Kras was used as an experimental test microorganism during the Apollo-Soyuz Test Project to study alternating vegetative mycelial and spore ring periodicity during space flight. Four cultures were launched in each of the spacecraft (Apollo and Soyuz). During the joint space-flight activities, two cultures from each spacecraft were exchanged. Selected duplicate cultures were maintained as controls in both the United States and the U.S.S.R. Spore ring morphology was periodically documented by photographing the specimens at approximately 12-hour intervals during the preflight, flight, and postflight periods of the experiment. A decreased growth-rate periodicity in the space-flight cultures was in part attributed to the reduced temperature in the spacecraft; however, one or more physical factors of space flight (weightlessness, etc.) may also have been involved. One of the four cultures grown in the reduced temperature environment of the Apollo spacecraft had a growth rate more rapid than that of the ground controls. Three of the space-flight cultures developed double spore rings during the period immediately after flight. This anomaly was attributed to the return to the Earth's gravity. The absence of spores in portions of one ring formed during space flight may have been caused by nutritional defects or media abnormality. Extensive studies will be required to determine the cause of this defect. There was no visible evidence of wedges in the cultures that would suggest naturally occurring or radiation-induced mutagenic alteration during space flight.

INTRODUCTION

The manifestation of various cyclic biological phenomena (biorhythms) of living organisms is in part or totally controlled by genetic, biochemical, and biophysical aspects. The occurrence of rhythmic events (biorhythms, circadian clocks, periodicities, oscillations, etc.) of man and other organisms has been a subject of interest and investigation for many years (refs. 14-1 to 14-4). Even though some space-flight studies have involved higher organisms having demonstrable biorhythms (refs. 14-5 to 14-8), there has been no previous investigation of the space-flight effects on the biorhythm or periodicity features of a microorganism. The opportunity to investigate this phenomenon in a microorganism was provided during the Apollo-Soyuz Test Project (ASTP) mission in July 1975.

Streptomyces levoris Kras exhibits a distinct periodicity of vegetative mycelial growth, which alternates with spore production. This growth characteristic results in a culture morphology in which uniform rings of mycelium and spores develop in an alternating pattern. When this microorganism is grown under known conditions using a defined biochemical medium and controlled temperature and light, increments of time

*This experiment was performed jointly and in collaboration with I. G. Akoev and associates of the Institute of Biophysics, U.S.S.R. Academy of Sciences, Pushchino, U.S.S.R. The joint experiment designation was AS-1.

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can be directly correlated with biological activity. Consequently, any deviation from the normal morphological pattern can be directly related to a specific time period. *Streptomyces levoris*, therefore, provided a suitable biological test system to investigate influences of space flight on the rhythms of vegetative and spore phase characteristics of both growth-rate periodicity and culture morphology during the ASTP preflight, flight, and postflight periods.

The Zone-Forming Fungi Experiment is one of the five experiments conducted as joint investigations by representatives of the United States and the U.S.S.R. The objectives of the U.S. participation in this experiment were (1) to study the effects of space flight on the biorhythms of *Streptomyces levoris* by comparing the growth-rate periodicity of the vegetative and spore phases within each culture, (2) to investigate the possible alteration of spore morphology and development by examination using the scanning electron microscope, and (3) to compare the effects of a 12-hour phase shift in growth periodicity features of the microorganism in cultures that were exchanged during the joint activities of the space flight. Additional studies were performed by and will be independently reported by U.S.S.R. investigators.

MATERIALS AND METHODS

Optimum growth of *Streptomyces levoris* (known as *Actinomyces levoris* Kras in the U.S.S.R.) was achieved by incubation at a temperature of 300 K (27° C) on an agar-based medium of inorganic salts, glucose, and yeast extract. Cultures were initiated by singlepoint inoculation of spores on the agar-based medium in 60- by 5-mm petri dishes 6 days preceding the ASTP flight. Phasing of the vegetative and spore production cycles was accomplished in both the United States and the U.S.S.R. by using a 12-hour light-dark period (100 to 200 lux) from 9:00 a.m. to 9:00 p.m. local time. The light-dark cycle was maintained until the cultures were selected for the experiment and placed in the experimental devices 4 days after inoculation.

When grown at a temperature of 300 K (27° C) on a defined medium, the first spore ring typically appears on the third day. Thereafter, one complete cycle of vegetative mycelium and subsequent spore phase generally occurs with a periodicity of 24 to 26 hours for 10 to 14 days. Because of nutritional depletion and metabolic waste diffusion within the media, the growth becomes slower after approximately 2 weeks.

A series of cultures from which the ASTP experimental specimens were selected was initiated 6 days before flight in both the United States and the U.S.S.R. at 9:00 a.m. local time at the respective laboratories. Because of logistics and certain preflight time-line constraints, it was necessary to select the cultures initiated in the United States and to place them in the devices 2 days before flight. The cultures initiated in the U.S.S.R. were selected and placed in the space-flight devices on the day of launch.

Two cultures of *Streptomyces levoris*, each contained in a 60- by 5-mm petri dish, were placed in each flight device (fig. 14-1). Two flight devices (four cultures) were prepared for launch in the

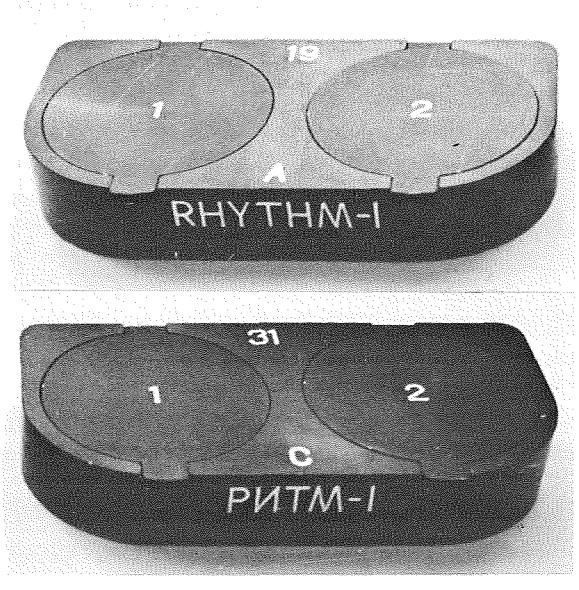

FIGURE 14-1.—Examples of the experiment devices. The "A" series was used by the United States and the "C" series by the U.S.S.R. Each device holds two petri dishes (60 by 5 mm), which are designated as "1" and "2."

Apollo spacecraft and two identical devices with matching cultures were prepared as U.S. ground controls. A similar procedure was followed by the U.S.S.R. representatives.

The main body of each device consisted of separable halves that were composed of anodized aluminum. A protective glass, fitted with a biologically nonreactive siliconized rubber ring, was inserted beneath the movable lid in the top half of each device. This glass served as a protective cover and enabled observation and photography of the cultures. The bottom half of the device, which included the petri dishes with cultures, spacing rings around the dishes, and siliconized rubber rings below the dishes, was fitted with the top half of the device to complete the assembly. The components of the experimental device are shown in figure 14-2. Radiation detectors of Lexan and cellulose acetate were placed beneath each petri dish as well as in the movable lid. The detectors would serve to evaluate visible points of alterations within the cultures in conjunction with particle tracks in the detectors. The bacteriophage T4Br+ was incorporated into an agar medium and dried to form a thin film. These films were placed in the flight devices beneath the lower layer of plastic dosimeters so that specific genetic alterations of the bacteriophage deoxyribonucleic acid, induced by the action of high-energy particles, could be studied (ref. 14-9). Data related to dosimetry and to the T4Br+ bacteriophage will be reported by the U.S.S.R. investigators.

Following assembly of the devices to be launched in the Apollo spacecraft, both the flight and control cultures were placed in a "Thermocont," or portable incubator, and were maintained

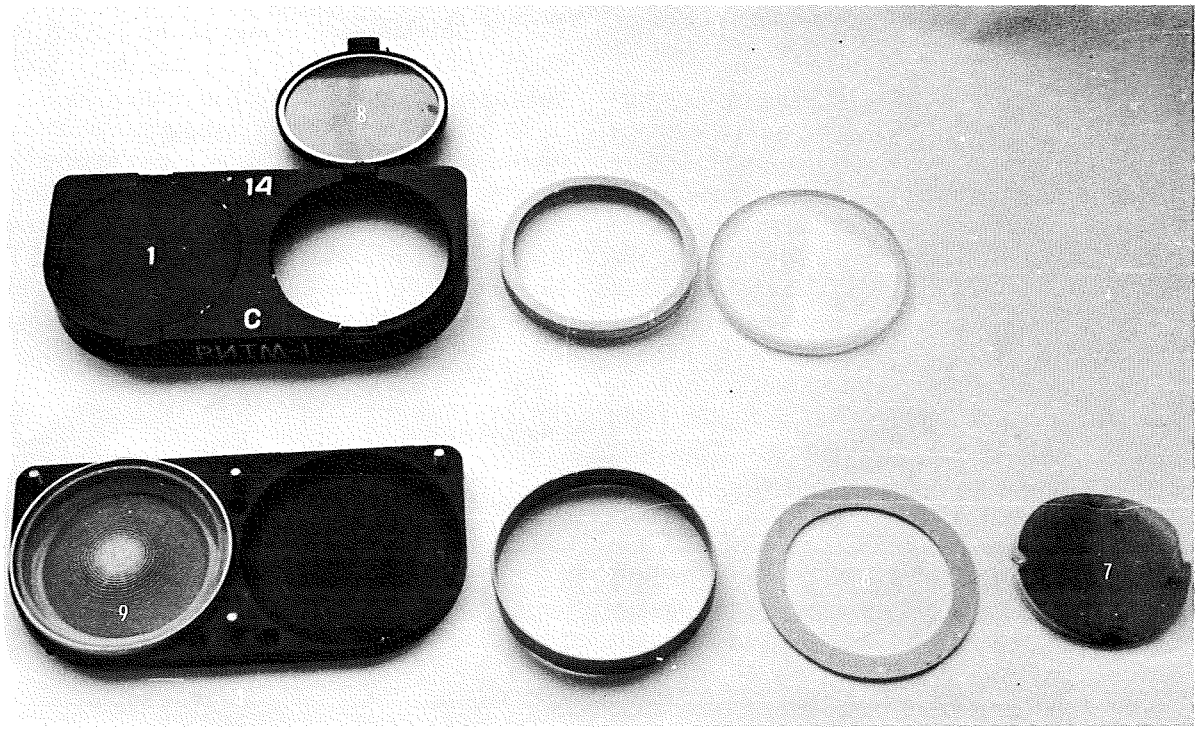

FIGURE 14-2.—Photograph of the components of a flight device. (1) Upper half of device with movable covers. (2) Siliconized rubber ring that fits around the protective glass. (3) Protective glass and rubber ring, which are inserted into the upper half of the device and serve to protect the specimen in the petri dish.(4) Bottom half of the device. (5) Metal spacing ring. (6) Siliconized rubber washer that fits in the well of the lower half of the device. (7) Radiation detector, which is positioned in the bottom of the device under the petri dish. (8) Radiation detector in the movable cover. (9) Petri dish and specimen.

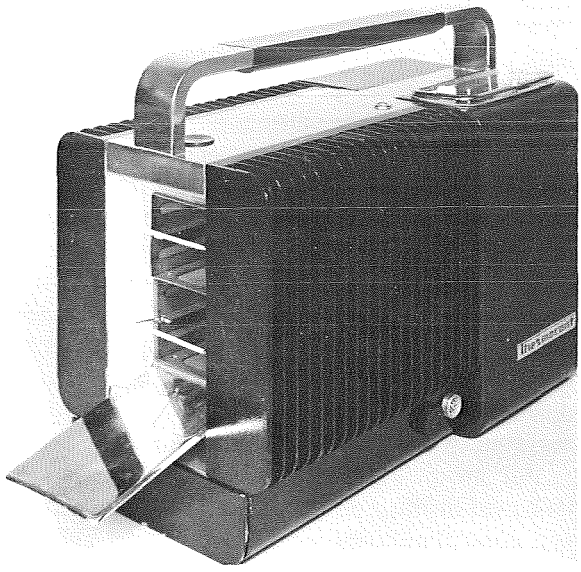

FIGURE 14-3.—The ac/dc portable incubator (transport container) "Thermocont" that was used to maintain cultures at a temperature of 300 K (27° C) during various phases of the experiment in the United States and the U.S.S.R.

at a temperature of 300 K (27° C) during transport to the launchsite (fig. 14-3). The control cultures were maintained in the incubator during the entire course of the experiment. Following recovery, the flight specimens were again placed in the portable incubator and maintained at a temperature of 300 K (27° C) until termination of the experiment. A similar procedure was followed by both laboratories.

Photography of the specimens was begun at the time the cultures were assembled in the flight devices. Both control and flight specimens were photographed at 12-hour (±3 hours) intervals until termination of the experiment. A Nikon 35-mm camera (fig. 14-4), equipped with a 55-mm lens (f/2.8) and adapted with a special framing device to facilitate focusing, was used for photography. The photographs were used to provide a comparative basis for growth analyses.

The U.S. experiment consisted of three time periods.

1. Preflight—Cultures were initiated on July 9, 1975. Specimens for the experiment were selected and placed in the devices on July 13. The control and flight specimens were transported from the NASA Lyndon B. Johnson Space Center (JSC) to the NASA John F. Kennedy Space Center (KSC), where the flight specimens were placed onboard the Apollo spacecraft on July 15, 1975.

2. In-flight—The flight specimens were launched from KSC on July 15 and were recovered at the splashdown site in the Pacific Ocean on July 24. On July 17, during the joint activity phase of the mission, space-flight crewmen of Apollo and Soyuz each exchanged one device containing two cultures (fig. 14-5). Following the Apollo launch, the controls were returned to JSC and then transported to the Apollo recovery site on July 21.

3. Postflight—Following recovery of the space-flight cultures on July 24, the flight devices were transported with the controls to JSC. From the time of recovery until the experiment was terminated on August 11, 1975, all specimens were maintained at a temperature of 300 K (27° C) in the portable incubator.

Similar time lines were followed by the U.S.S.R. investigators. However, the U.S.S.R. in-flight period was July 15 to 21 and the U.S.S.R. postflight period was July 21 to August 16.

To facilitate preliminary comparison of data in the respective periods, August 1 was selected as the cutoff date for the postflight period, although the experiment was continued until August 11 in the United States and August 16 in the U.S.S.R. On August 29, following termination of the experiment, the Thermocont and specimens from

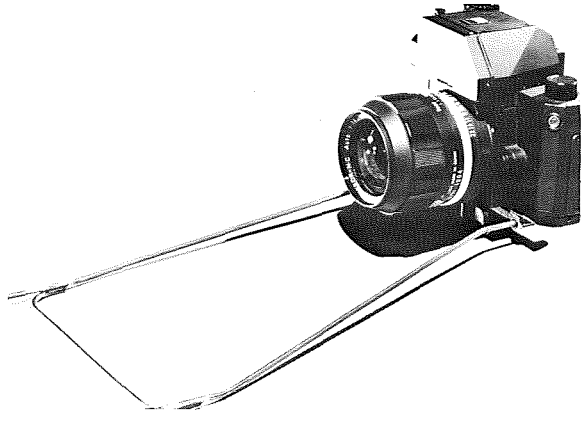

FIGURE 14-4.—Photographic apparatus used in the United States during both ground-support and Apollo in-flight photography of the experiment.

FIGURE 14-5.—Photograph of ASTP crewmen exchanging the culture devices. The exchange occurred on July 17, 1975.

the United States were shipped to the laboratory in the U.S.S.R. On October 7, the radiation detectors were marked for position and were removed from the flight and control devices in the presence of science representatives of both the United States and the U.S.S.R.

RESULTS AND DISCUSSION

The general scheme of the experiment was performed according to the project outline. All the flight and control cultures of *Streptomyces* exhibited growth during the preflight, flight, and postflight periods of the experiment without apparent contamination.

Cultures were initiated July 9 for the experiment in both the United States and the U.S.S.R. At the respective launch times on July 15, the rate of vegetative growth and the subsequent spore ring development (biorhythm) were different. The difference in growth rate and frequency between the two sets of cultures continued throughout the experiment.

The total number of spore rings formed by the control and flight cultures at the end of each of the three periods is shown in table 14-I. Since there was a 3-day difference between the U.S. and U.S.S.R. in-flight time lines, the average growth rate per day for each culture during each experiment period is also shown. The latter value provides a more uniform basis of comparison between cultures.

The controls cultured in the United States grew at the same rate when compared with each other within a given time period, with the exception of culture number 2 of device A-25, which exhibited a slight decrease in growth rate during the postflight period. (See table 14-I.) During the preflight period, the flight cultures (A-19 and

A-22) had growth rates identical to those of the controls. At the end of the flight period, the flight specimens were noticeably different from the controls in growth rate and frequency.

The temperature profile in the Apollo spacecraft is shown in figure 14-6. Even though the reduced and variable temperature would cause a decreased growth rate, this factor alone does not explain the difference observed in the flight cultures during the flight period. The growth rate of the in-flight cultures would have decreased uniformly had temperature been the only factor involved. One or more physical factors of space flight (weightlessness, magnetic field forces, and possible solar effects) appear to have invoked various effects on the growth-rate periodicity, because the space-flight cultures exhibited different types of growth patterns and biorhythms. Some of the more apparent differences are (1) increased growth rate of number 2 of A-22, which grew more rapidly during flight at a reduced temperature compared to the other space-flight cultures or the controls, (2) differences in reduced growth rate of space-flight

TABLE 14-I.—Growth-Rate Comparison of Streptomyces Levoris During ASTP

Status	*Device*	*Culture*	*Preflight period*[a]		*Flight period*[b]		*Postflight period*[c]	
			Number of rings[d]	*Growth rate*[e]	*Number of rings*[d]	*Growth rate*[e]	*Number of rings*[d]	*Growth rate*[e]
United States								
Control	A-24	1	4.0	1.0	9.5	0.61	13.0	0.44
		2	4.0	1.0	9.5	.61	13.0	.44
	A-25	1	4.0	1.0	9.5	0.61	13.0	0.44
		2	4.0	1.0	9.5	.61	12.5	.38
Flight	A-22	1	4.0	1.0	8.0	0.4	12.0	0.5
		2	4.0	1.0	10.0	.66	15.0	.63
	A-19[f]	1	4.0	1.0	7.0	0.5	13.0	0.55
		2	4.0	1.0	7.0	.5	13.0	.55
U.S.S.R.								
Control	C-35	1	3.0	0.75	6.1	0.52	9.5	0.31
		2	3.0	.75	6.1	.53	9.3	.29
	C-36	1	2.0	0.5	6.0	0.67	9.2	0.29
		2	2.0	.5	6.2	.7	11.5	.48
Flight	C-31	1	3.0	0.75	5.0	0.33	9.3	0.39
		2	2.8	.7	4.8	.33	8.5	.33
	C-12[g]	1	2.5	0.63	7.3	0.53	10.3	0.38
		2	2.3	.58	6.3	.44	9.2	.36

[a]U.S. and U.S.S.R. preflight period—July 9 to 15.
[b]U.S. flight period—July 15 to July 24; U.S.S.R. flight period—July 15 to July 21
[c]U.S. postflight period—July 24 to August 1; U.S.S.R. postflight period—July 21 to August 1.
[d]Total number of rings at the end of each period.
[e]Number of spore rings formed per day during each period.
[f]Exchanged from Apollo to Soyuz on July 17.
[g]Exchanged from Soyuz to Apollo on July 17.

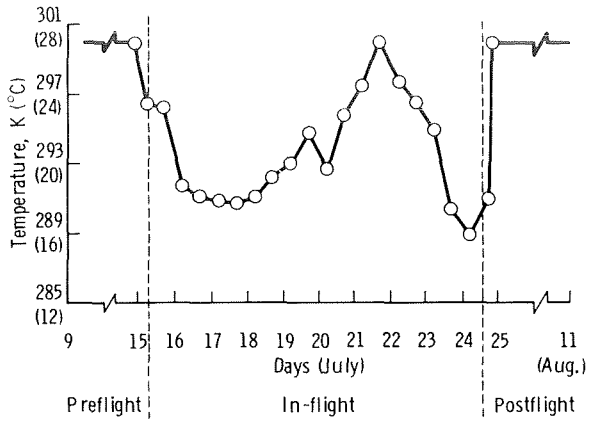

FIGURE 14-6.—Temperature profile in the Apollo spacecraft during the ASTP mission. Each point represents the average temperature for a 12-hour period.

cultures maintained at the same temperature (e.g., no. 1 of A-22 as compared with no. 2 of A-22, no. 1 of C-12 as compared with no. 2 of C-12), and (3) differences between the flight cultures of each group (A-22 and A-19, C-31 and C-12). Direct comparison of flight cultures was made difficult by the exchange of devices during flight.

Spore morphology during the flight period was distinct and, in general, exhibited no alteration of growth pattern. Spores failed to form in two distinct areas of the last in-flight spore ring of A-19 culture number 1 (fig. 14-7). At first, it was suspected that this void area in the spore ring was the result of radiation effects; however, in a postflight radiation experiment in which *Streptomyces* cultures were subjected to neon ions at radiation dose levels of 1 to 100 J/kg (100 to 10 000 rads), not a single aberration of this nature was observed. No other explanation for this type of aberration is suggested at this time, especially in view of the fact that a number of variables are known to have been involved in comparisons of the spaceflight cultures with each other or with ground controls.

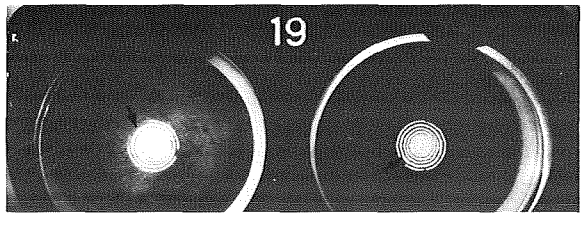

FIGURE 14-7.—Photograph of culture A-19. The last in-flight spore ring in which spores failed to form is indicated by arrows.

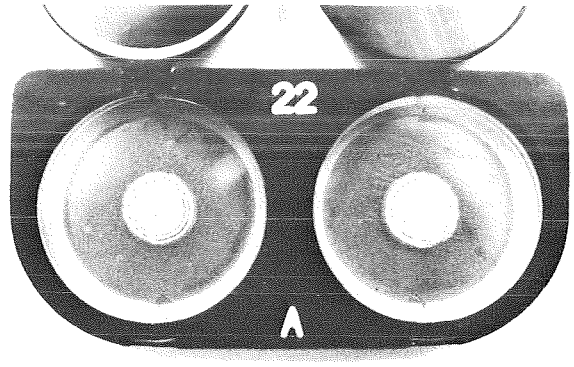

FIGURE 14-8.—Photograph of culture A-22 showing spore ring offset and subsequent spiral ring formation.

Spore ring offsets and subsequent spiral ring formation (fig. 14-8) also occurred during the flight period in number 2 of A-22. Similar offsets and spirals were observed in the ground controls.

Unusual double spore rings were formed during the period immediately after flight in cultures of device A-19 and in culture number 2 of device C-31 (fig. 14-9). Although such formations may be coincidental, it is also possible that the dynamics of physical forces related to reentry and landing of

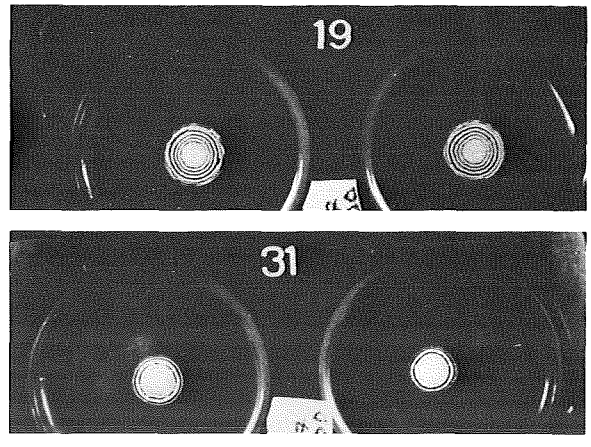

FIGURE 14-9.—Photographs of cultures A-19 and C-31 showing double spore rings formed during the period immediately after flight.

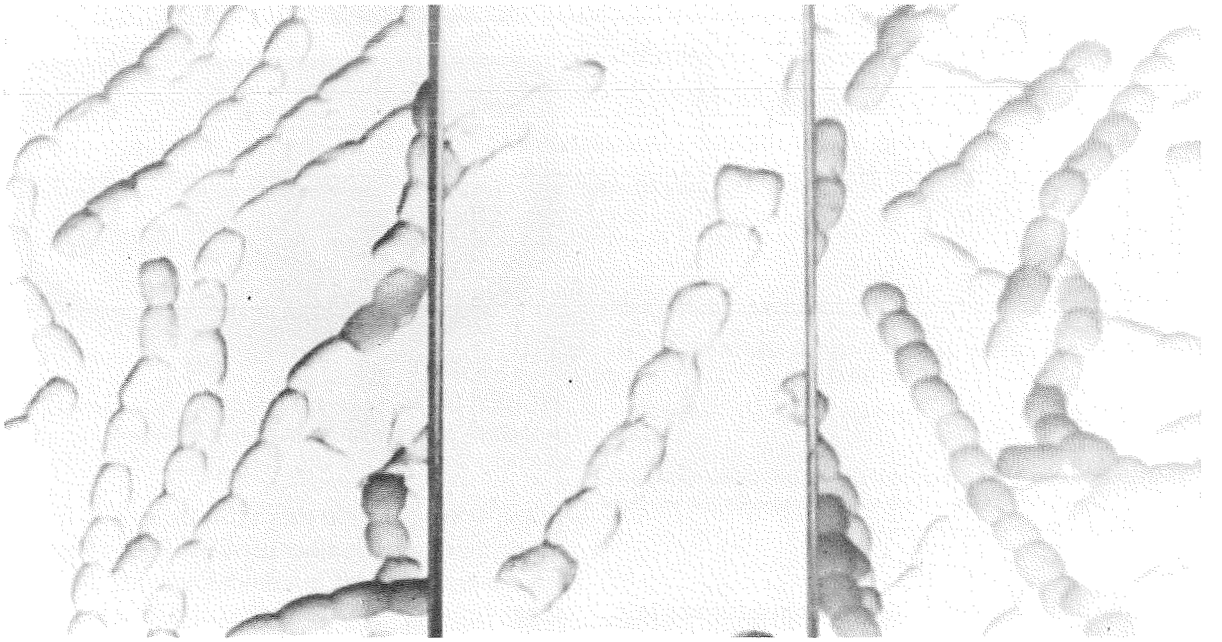

FIGURE 14-10.—Scanning electron micrographs of representative spores from control cultures.

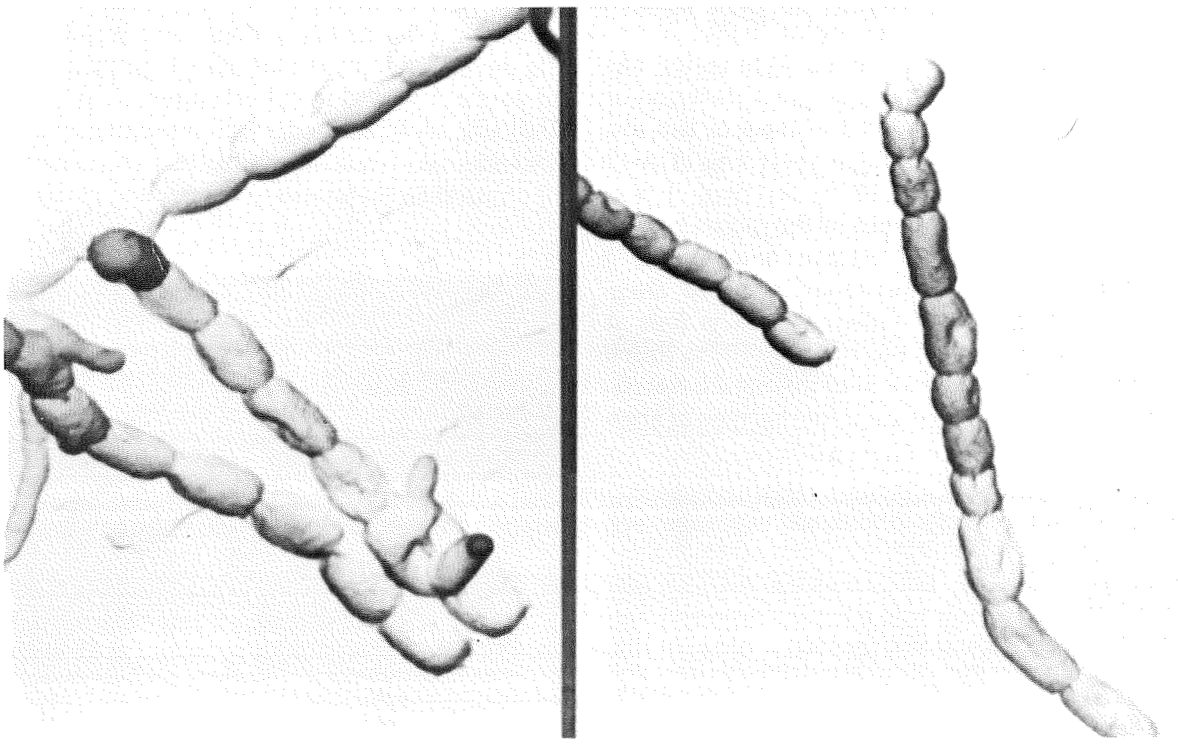

FIGURE 14-11.—Scanning electron micrographs of irregular and elongated spores from spore rings formed during flight in culture 2 of A-22.

the Soyuz spacecraft may have contributed to the growth pattern aberrations. The subsequent spore rings in the cultures developed in a normal pattern, and this type of aberration was not observed in the Apollo flight specimens.

Postflight subculturing of the U.S. and the U.S.S.R. control and space-flight cultures revealed little or no difference in the growth rates of spore ring formation when grown on the standard culture medium.

Before inoculation of cultures for the space-flight experiment, an attempt was made to standardize procedures and components of the nutritional medium; however, specific chemicals were not obtained from the same sources in all cases. The differences in growth rate observed between the two sets of cultures (U.S. and U.S.S.R. initiated cultures) were probably due to a slight difference in the medium rather than an inherent biorhythm difference between the source cultures for the experiment.

Preliminary examination with a scanning electron microscope revealed an irregular and elongated spore morphology in a portion of both the flight cultures when compared with the controls (fig. 14-10). Spores of number 2 of A-22 exhibited a greater deviation from the normal spore morphology than did any of the other space-flight cultures (fig. 14-11); however, spores that formed during the postflight period appeared similar to those of the controls. Investigations are in progress to determine if this abnormal spore morphology will appear in subcultures.

SUMMARY

Cultures (U.S. device A-19 and U.S.S.R. device C-12) were exchanged on July 17 during the ASTP joint activities phase. Because the respective cultures had been initiated on July 9 at 9:00 a.m. local time in both the United States and the U.S.S.R., there was a 12-hour time differential (time phase shift). The purpose of exchanging one set of cultures during the space flight was to observe the effect, if any, of the time differential relative to growth-rate biorhythm. Because of the diversity of growth-rate patterns exhibited by the flight specimens, it was not possible to determine whether the time phase shift affected the biorhythm in the exchanged flight specimens. (See table 14-I.)

There is an ordered oscillation of cellular events that is visibly manifest in *Streptomyces levoris* and that results in the intrinsic rhythm of the vegetative and spore phases. It is also important to keep in mind that within the context of this experiment, the temperature, nutritional medium factors, and light conditions could have contributed to the overall results.

Although differences were observed in the biorhythm of *Streptomyces levoris* during space flight, the differences were not uniform. Furthermore, because all cultures did not react in the same manner during flight and because there were distinct preflight frequency differences between the two groups of cultures, statistical analysis of the data was not possible.

It appears that the single most variable factor related to the experiment was lack of temperature control for the space-flight specimens. Although the general temperature profile can be repeated on subcultures under laboratory conditions, the influence of weightlessness and other space-flight factors cannot be reconstructed to provide a meaningful analysis for correlative purposes.

The results obtained from this experiment indicated that space flight did affect the biorhythm of *Streptomyces levoris* vegetative and spore phases. However, the extent to which these cultures were affected cannot be accurately determined because of the action of such variables as temperature, nutritional medium, and the physical force factors associated with the launch and recovery process.

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